

CME Differential mechanisms of action of interferon- β and glatiramer acetate in MS

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Abstract—Interferon- β and glatiramer acetate (GA) are the two main groups of drugs used in the treatment of MS. Notably, while both ultimately decrease CNS inflammation, they do so by very different mechanisms. Interferon- β has potent activity at the blood-brain barrier and impairs the trafficking of inflammatory cells into the CNS. In contrast, GA has negligible effect at the blood-brain barrier, allowing GA-specific T helper 2 lymphocytes to enter the CNS to decrease inflammation through bystander suppression. Other differences are also emphasized. The presence of GA-reactive lymphocytes within the CNS parenchyma may have the additional benefit of conferring neuroprotection through protective autoimmunity.

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The introduction of interferon- β (Betaseron, Avonex, and Rebif) and glatiramer acetate (GA; Copaxone) into MS therapeutics has altered the natural course of the disease. The annualized relapse rate of drug-treated patients is lower compared to placebo controls, and more treated patients remain relapse free for several years relative to untreated cohorts. What is still unclear is how interferon- β and GA achieve their therapeutic benefit in MS. This review discusses the possible mechanisms by which interferon- β and GA may work in MS and emphasizes their different modes of activity. All three interferon- β preparations are used interchangeably here, given that the evidence does not suggest their activity to be different if utilized at similar concentrations, at least in vitro.

Mechanisms of action of drugs in the periphery.

Antigen presentation and cytokines. A key concept in immunology required to discuss this section is the phenomenon of antigen presentation. Here, a foreign molecule is first engulfed by an antigen presenting cell (APC), which is usually a dendritic cell, macrophage, or B cell. Part of that antigen is then displayed on the surface of the APC within the groove of a major histocompatibility complex (MHC) molecule. The antigen-MHC complex is recognized by a specific T-cell receptor (TCR) of a responding T cell. Costimulatory molecules are also required to provide optimal activation, including CD40 interacting with CD40L on APC, and B7 with CD28 on T cell. If the costimulatory molecules are not engaged, the responding T cell may undergo functional inactivation (anergy) or apoptosis. Upon antigen

presentation, the T cell then undergoes clonal expansion and differentiation into effector cells. A subtype of T cells with the CD4 molecule on its surface differentiates into two subsets: T helper 1 (Th1), and T helper 2 (Th2) cells (figure 1). The Th1 and Th2 effector arms have important functions, including promoting cell-mediated immunity (Th1) and humoral immunity (Th2). Th1 cells produce Th1 cytokines that include interleukin (IL)-2, IL-12, interferon- γ , and tumor necrosis factor (TNF)- α ; in general, these tend to be proinflammatory. Th2 cells produce Th2 cytokines such as IL-4, IL-5, and IL-10, which tend to be anti-inflammatory (regulatory); indeed, Th2 cytokines can inhibit the production of cytokines by Th1 cells or macrophages. In MS, there appears to be an elevation of Th1 cytokines, and a diminution of Th2 cytokines, preceding and during relapse.^{1,2} Finally, a current concept of MS pathogenesis is that myelin-reactive CD4⁺ Th1 cells are activated and these then traffic into the CNS to produce disease.¹⁻⁴ Thus, some of the aims of therapy would be to decrease the generation/activation of autoreactive Th1 cells through antigen presentation, and to produce “immune deviation” away from a Th1 milieu toward a Th2 environment (see figure 1).

GA and interferon- β both affect antigen presentation and cytokine levels but by different means. GA and interferon- β both affect antigen presentation and the cytokine milieu, but they do so by different mechanisms (figure 2). GA has high affinity for the MHC groove and is thought to bind to, and to be displayed, as an antigen within this

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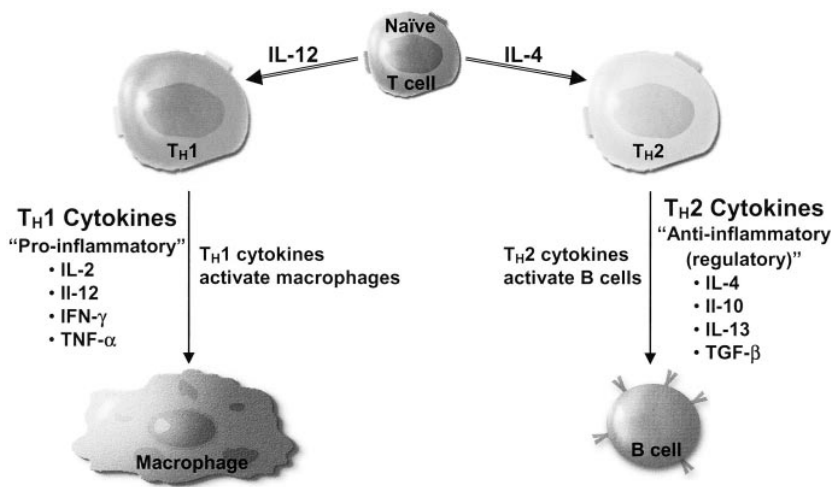


Figure 1. Naive CD4 helper T cells differentiate into T helper 1 (Th1) and T helper 2 (Th2) cells. Th1 cells are proinflammatory, whereas Th2 cells are anti-inflammatory or regulatory. Evidence suggests a preponderance of Th1 cytokines in MS relapse, and elevated Th2 cytokines during remission. Thus, rational therapies include strategies to decrease the generation of autoreactive Th1 cells, or immune deviation in favor of a Th2 environment.

groove.⁵ Alternatively, GA is engulfed by APC and fragments are then presented. Either way, the presentation of GA leads to the generation of GA-specific T cells. Through mechanisms that are still unclear, the GA-specific T cells are predominantly Th2 biased, as has been amply demonstrated in animal studies and in leukocytes derived from individuals with MS treated with GA.^{5,6} That the generation of GA-specific Th2 cells is important is indicated by the finding that the injection of these cells into mice prevented experimental autoimmune encephalitis (EAE) when animals were subsequently immunized with spinal cord homogenates.⁷ EAE is an inflammatory disease in animals that bears several histologic features of MS.

Interferon- β also affects antigen presentation, but by decreasing the expression of molecules that are necessary for this process (see figure 2). Thus, interferon- β is particularly effective in preventing the interferon- γ -induced upregulation of MHC II on APC.¹ Interferon- β also downregulates the expression of co-stimulatory molecules, and impacts on other aspects of antigen presentation. As an antiproliferative agent, interferon- β inhibits the expansion

of T cell clones. Recently, interferon- β (and GA) was shown to decrease the production by dendritic cells of IL-12, which is required for differentiation along the Th1 route.⁸ Finally, interferon- β inhibits the expression of FLIP, an anti-apoptotic protein, leading to an increased incidence of death of T cells.⁹ Overall, when the frequency of myelin basic protein (MBP) reactive T cells was analyzed in MS, this was found to be reduced following treatment with interferon- β compared to pretreatment levels.¹⁰ In contrast, GA-reactive T cells may have a survival advantage.¹¹

Does interferon- β cause a Th2 shift as is the case for GA? The literature has been extremely confusing. Several studies have reported the elevation of the Th2 cytokine, IL-10, in the mononuclear cell fraction, and serum and CSF of patients with MS treated with interferon- β ,^{12,13} but this has not been confirmed in other studies.¹⁴ Furthermore, while the Th1 cytokines, interferon- γ , IL-12 and TNF α were decreased by interferon- β in a majority of studies,¹ no difference, or even an increase,^{15,16} were noted by others. In other studies, the number of circulating T cells that express either Th1 or Th2 cytokines was reduced following interferon- β therapy, indicating a

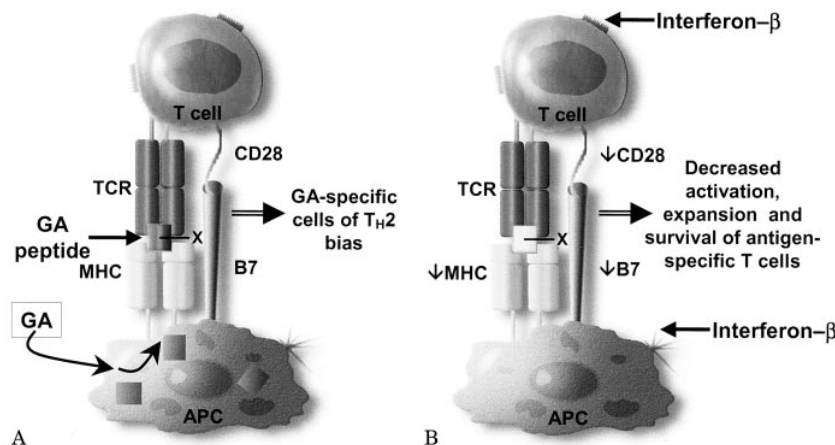


Figure 2. Contrasting the mechanisms of glatiramer acetate (GA) and interferon- β on antigen presentation. In panel A, the high affinity of GA for the major histocompatibility complex groove or the uptake of GA by an antigen presenting cell (APC) leads to the presentation of GA as an antigen and the generation of GA-specific cells that are T helper 2 biased. In the case of interferon- β , which acts on its receptor on T cells and APC, this decreases the expression of molecules needed for antigen presentation. Together with a further activity of interferon- β on T cell expansion and survival, this leads to the decreased generation of antigen-specific T cells. In both panels, x refers to an antigen that sits on the MHC groove.

more general suppression of both subsets.¹⁷ Differences in experimental conditions, patient sampling, and duration of treatment may have affected the outcome of results. Overall, however, the majority of studies do indicate a suppression of the generation of autoreactive CD4⁺ Th1 cells and decrease of Th1 cytokines following interferon- β treatment, but a clear deviation away from a Th1 milieu to a Th2 environment has been difficult to demonstrate.

In summary, both GA and interferon- β alter antigen presentation and the cytokine milieu but by different mechanisms (see figure 2). GA leads to the formation of GA-specific Th2 cells with immunoregulatory properties, whereas interferon- β inhibits several aspects of antigen presentation that leads to the generation and expansion of autoreactive T cells. The net result of both treatments in the periphery is the decrease of a proinflammatory milieu.

Other mechanisms in the periphery. Although the above discussions have focused on the CD4 subset of T cells, the CD8 suppressor/cytotoxic T cells may also be altered. Particularly, GA therapy upregulates the CD8 responses and restore these to levels observed in healthy individuals;¹⁸ a subset of CD8 T cells is thought to have regulatory roles in MS and EAE.

Finally, since CD4⁺ Th2 cells activate B cells to produce immunoglobulins (see figure 1), it is reasonable to address whether the latter is elevated in patients with MS on GA therapy. Indeed, in a study of 130 patients on GA treatment, all developed GA-specific antibodies that peaked at 3 months after initiation of treatment. The role, if any, of these antibodies in mediating the beneficial actions of GA is unclear; however, these antibodies did not appear to negate clinical activity.¹⁹ A large literature exists on the generation of antibodies to interferon- β , and the field remains divided as to the significance of these antibodies.

Mechanisms of action of drugs at the blood-brain barrier. *Trafficking of inflammatory cells into the CNS.* The migration of activated T cells into the CNS is critical to initiating and sustaining the pathology of MS.³ In correspondence, areas of CNS demyelination or axonal loss contain high numbers of various inflammatory cell types.²⁰ Thus, it is logical to address whether interferon- β and GA impact on the influx of inflammatory cells into the CNS.

For T cells to infiltrate into the CNS, a number of events are necessary. First, adhesion molecules on T cells interact with their counter receptors on endothelial cells. These ligand pairs include the integrins very late activation antigen-4 (VLA-4) and leukocyte function antigen (LFA-1) on T cells, and vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) on endothelial cells. In general, these molecules are upregulated in MS. Second, there is the expression of chemokines, which provide a directional gradient for leukocytes to enter

the CNS, and also increase the affinity of integrins (e.g., VLA-4) for their counterligands on endothelial cells. Specific chemokines (e.g., IP10, MIG, and RANTES) are present in MS lesions, and their corresponding receptors are upregulated on circulating T cells.^{21,22}

Once past the endothelial barrier, the leukocyte encounters a barrier of extracellular matrix (ECM) proteins present within the basement membrane. Transit across the ECM barrier into the CNS parenchyma appears to require the coordinate action of proteolytic enzymes including the matrix metalloproteinases (MMP).²³

Interferon- β inhibits the trafficking of T cells but GA does not. Interferon- β has profound activities on several components of the process required for the migration of inflammatory cells into the CNS. At the level of adhesion molecules, interferon- β decreases their expression. Many cell-anchored adhesion molecules are processed into a soluble form, which then interacts with receptors on T cells, preventing the latter from interacting with endothelial cells. The conversion of cell-associated VCAM-1 into soluble VCAM-1 is facilitated by interferon- β .²⁴ Coordinately, these mechanisms decrease the ability of T cells to adhere on the endothelium.

At the level of chemokine and chemokine receptor expression, interferon- β treatment decreases the expression of several chemokines and the CCR5 receptor,^{25,26} although this was not confirmed.¹⁷ These actions should decrease the chemokine gradient that facilitates the entry of cells into the CNS.

With respect to MMP, the production of MMP-9 by activated T cells is decreased by interferon- β , and this corresponded with a decrease in the capacity of T cells to transmigrate across a matrix barrier.^{27,28} Interferon- β treatment of patients with MS results in the decrease of the serum content of MMP-9²⁹ and of the number of mononuclear cells that express various MMP members.³⁰ More recently, mononuclear cells from patients with MS on interferon- β treatment for 12 months were found to have reduced MMP-7 and MMP-9 transcript levels; moreover, this occurred only in patients with relapsing remitting but not secondary progressive MS.³¹ The sum of the above activities of interferon- β is the reduction in the number of inflammatory cells that infiltrate into the CNS (figure 3). This is borne out in EAE where interferon- β treatment decreases the number of infiltrates in afflicted animals.³²

In contrast to interferon- β , GA does not appear to affect the transmigration of leukocytes into the CNS (see figure 3). Thus, GA treatment does not alter the expression of adhesion molecules on cultured endothelial cells³³ and does not affect MMP production by leukocytes (Giuliani and Yong, manuscript in preparation). High concentrations of GA applied to glioma cells in vitro block the cytokine-induced production of the chemokine RANTES,³⁴ but the importance of this to lymphocytes is unclear. In correspondence with the lack of activity of GA on molecules that

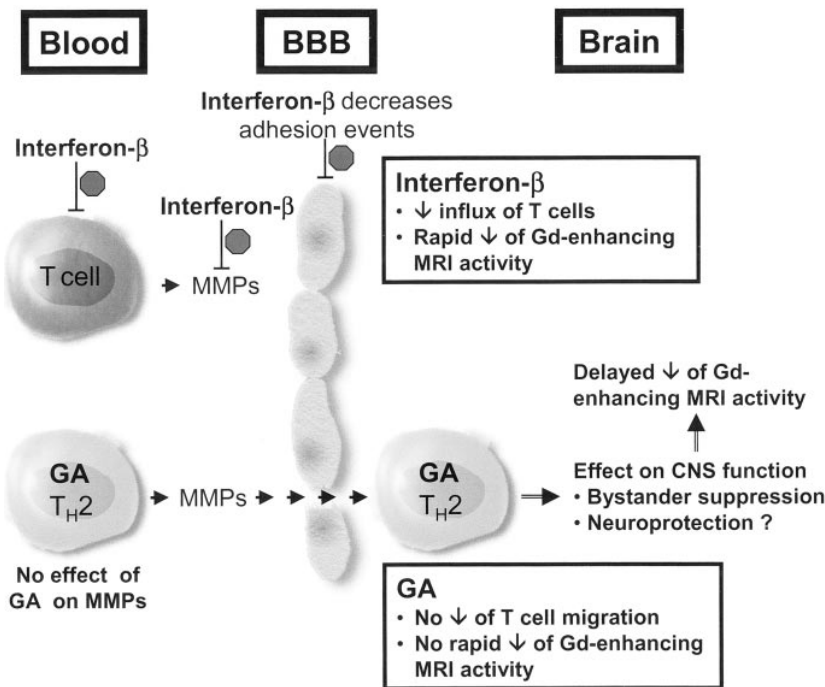


Figure 3. Contrasting the effects of interferon-β and GA at the blood-brain barrier and within the CNS. Interferon-β decreases the production of matrix metalloproteinases by T cells and also affects the adhesion of T cells onto the endothelium. These reduce the influx of T cells into the CNS and lead to a rapid resolution of Gd-enhancing MRI activity. In contrast, GA-specific T helper 2 cells traffic into the CNS to produce bystander suppression and possible neuroprotection. The lack of action of GA in excluding T cell infiltration does not produce a rapid decrease of Gd-enhancing MRI activity. Rather, due to actions within the CNS, a delayed reduction of MRI activity results.

affect transmigration, Th2 polarized GA-reactive cells traffic readily into the CNS of EAE-afflicted animals.³⁵

Does the differential activity of interferon-β and GA at the blood-brain barrier account for the observed clinical differences in MRI activity? A striking finding in patients with MS who are initiated on interferon-β therapy is the rapidity of resolution of the gadolinium (Gd)-enhancing activity on MRI.³⁶ In contrast, Gd-enhancing MRI activity is decreased more gradually in patients on GA.³⁷ Gd-enhancing MRI activity is correlated with lymphocyte infiltration and increase of MMP levels leading to blood-brain barrier (BBB) disruption.^{38,39} The multiple ways by which interferon-β decreases lymphocyte infiltration, and especially its reduction of proteolytic MMP levels, likely accounts for its rapid resolution of Gd-enhancing MRI activity (see figure 3).

In contrast, by not having any direct action on MMP or lymphocyte infiltration, GA does not produce a rapid resolution of Gd-enhancing MRI activity. It is important to point out that GA-reactive Th2 cells should have entered the CNS early in treatment to begin to achieve their effects within the CNS (discussed below). Over the longer term, the resolution of the Gd-enhancing MRI activity would be a reflection of the activity of GA on elements within the CNS, which then helps repair the BBB from within the CNS (see figure 3). These observations caution against the mere use of Gd-enhancing MRI signatures to document the effectiveness of a particular therapy for MS. The lack of acute effects on the BBB does not equate with lack of efficacy, if other modes of action predominate for such drugs.

In summary, interferon-β exerts multiple actions

at the BBB to exclude leukocytes from entering the CNS, but GA does not. This major differential may help account for the finding that interferon-β rapidly resolves Gd-enhancing MRI activity, while GA does not (see figure 3).

Mechanisms of action of drugs within the CNS. *Bystander suppression as a mechanism for GA within the CNS.* By virtue of excluding cells from entering the CNS parenchyma, and because interferon-β itself is not thought to enter the CNS, it seems prudent to state that interferon-β has no direct activity within the brain and spinal cord. Thus, the resolution of CNS inflammation by interferon-β could be considered indirect, since this would be the result of inhibiting the infiltration of inflammatory cells into the CNS.

In contrast, GA-polarized Th2 cells enter the CNS³⁵ and, within the CNS, are thought to decrease CNS inflammation by a phenomenon described as bystander suppression. In this regard, GA-specific Th2 cells within the CNS become reactivated by antigen presentation through cells that are likely microglia or macrophages that have infiltrated the CNS. Here, the antigen that is presented by microglia/macrophages is likely not GA, since this drug is rapidly metabolized following administration and is not thought to enter the CNS. There is evidence to indicate that GA-specific T cells recognize a variety of antigens presented by APC.⁴⁰ For instance, GA-specific T cells from animal studies proliferate in response to various myelin proteins *in vitro*;⁷ indeed, GA was originally designed to simulate the structure of MBP. Thus, it is likely that the presentation of degraded myelin components by microglia or macro-

Table Similarities and contrasts of the mechanisms of interferon- β and GA in MS

Biology	Interferon- β	GA
Antigen presentation		
Decreased expression of MHC II expression	Yes	No
Reduced level of costimulatory molecules	Yes	No
Inhibition of clonal expansion of autoreactive T cells	Yes	Yes
Increased apoptosis of autoreactive T cells	Yes	Not clear
Decrease of proinflammatory cytokines	Yes	Yes
Th1 to Th2 deviation	Not clear	Yes
Leukocyte trafficking across the BBB		
Decreased expression of adhesion molecules	Yes	No
Inhibition of chemokine expression	Yes	No
Inhibition of MMPs	Yes	No
Excludes leukocytes from entering the CNS	Yes	No
Events within the CNS		
Bystander suppression	No	Yes
Direct neuroprotection	Not clear	Possibly

GA = glatiramer acetate; MHC = major histocompatibility complex; BBB = blood-brain barrier; MMP = matrix metalloproteinase.

phages within the CNS leads to the reactivation of GA-specific T cells. The expansion of these Th2 polarized cells within the CNS results in the release of anti-inflammatory cytokines, which then impair the expansion of myelin autoreactive T cells that are within the CNS. This is bystander suppression. Indeed, the phenomenon of bystander suppression means that GA may be potentially useful in other autoimmune diseases where Th1 cells predominate.

Protective autoimmunity and GA. In contrast to the commonly accepted idea that autoreactive T cells produce the CNS pathology in MS, recent evidence demonstrates that some autoreactive T cells have neuroprotective functions. This was first demonstrated in an optic nerve crush model in rats, which produced loss of retinal ganglion neurons. If animals were injected with MBP-reactive T cells immediately after the crush injury, which led to these cells accumulating in the injured optic nerve, the subsequent loss of retinal ganglion neurons was attenuated.⁴¹ The neuroprotective effect of T cells has also been observed after spinal cord injuries.⁴² These results have generated much interest in the idea of protective autoimmunity, whereby autoreactive T cells protect against the loss of axons or neurons. Thus, it may be disadvantageous to limit the entire T cell immune response in the CNS following injury.

The mechanism(s) by which autoreactive T cells alleviate injury is still unclear, but it has become

evident that T cells, B cells, and macrophages secrete a variety of neurotrophic factors.⁴³ Indeed, brain-derived neurotrophic factor was localized by immunohistochemistry to inflammatory infiltrates in MS lesions⁴³ and in EAE.⁴⁴ Thus, the increased availability of neurotrophic factors resulting from the infiltration of inflammatory cells may attenuate injury. Alternatively, neurotrophins have many beneficial immunoregulatory functions and this may favor a reduction of the undesirable effects of CNS inflammation.⁴⁵

It is obvious that much remains to be done to define whether, when, and how autoreactive T cells are beneficial or detrimental to CNS recovery. Where myelin reactive T cells were used to alleviate neuronal loss after a traumatic injury to the optic nerve or the spinal cord,^{41,44} symptoms of EAE appeared in animals. It is unlikely that myelin reactive T cells would be used to alleviate injury in MS, but can other more benign T cell lines be used? Specifically, does the generation of GA-specific T cells that enter the CNS provide for neuroprotection? In this regard, it is noteworthy that when rats were subjected to an optic nerve crush, and then immediately injected with GA-specific T cells, the number of surviving retinal ganglion neurons after 2 weeks was higher in treated animals compared to injured controls.⁴⁶ More recently, using a model where the intraocular injection of glutamate in mice destroyed retinal ganglion neurons, the toxicity of glutamate was alleviated in mice immunized with GA, but not with MBP or myelin oligodendrocyte glycoprotein.⁴⁷ Collectively, these results suggest that in patients with MS treated with GA, the GA-reactive Th2 cells that enter the CNS have the potential to provide neuroprotection. Indeed, in an MRI study evaluating the proportion of new MS lesions that evolved into 'black holes,' which are thought to represent lesions where severe tissue disruption has occurred, the proportion was lower in GA-treated compared to placebo patients after 7 and 8 months of therapy.⁴⁸

In summary, both interferon- β and GA decrease CNS inflammation but by different means (see figure 3). Interferon- β excludes inflammatory leukocytes from the CNS, whereas GA-reactive Th2 cells enter the CNS to dampen neuroinflammation through bystander suppression. The presence of GA-reactive cells within the CNS may confer protective autoimmunity and alleviate the loss of CNS tissue. It is likely that interferon- β treatment also decreases CNS neuronal and axonal loss, but the mechanism for this would be indirect, i.e., by the exclusion of the largely pathogenic inflammatory cells that enter the CNS, even at the expense of excluding some protective lymphocytes.

Combination therapy? It is logical to ask whether GA and interferon- β can be combined to increase their effectiveness. Until the results of a formal combination trial are available, the answer is not immediately apparent, and is dependent on what the pivotal mechanism of each drug would ultimately prove to be. Thus,

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